THE DEVELOPMENT OF GREGARINES AND THEIR RELATION TO THE HOST TISSUES: (I) IN *STENOPHORA LACTARIA* WATSON *

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The object of this paper is the depiction of the stages through which the sporozoite passes in the species *Stenophora lactaria* Watson in becoming a free adult sporont. Whether or not the trophozoite at any stage in its development possesses an epimerite and the effect of the parasite upon the cell parasitized will also be discussed, conclusions reached affecting only the particular species under consideration. This effect upon cells parasitized will be reported in several other genera before conclusions can be stated as to the general influence upon the host-cells.

Stages in the growth of the parasite from the sporozoite to the sporont have been described by many writers. Léger and Duboscq have studied the development of Pyxinia (1902), Stylorhynchus (1904), and Pileocephalus (1909a), and to some extent of Stenophora (1904); Laveran and Mesnil of Gregarina (1900); and Siedlecki, Brasil, Caullery and Mesnil, and Hesse of parasites in the tunicates and annelids. In very few instances has a complete series of stages been shown.

To the writer's knowledge, consecutive stages from the incipient penetration to the vacation of the cell by the parasite have not been depicted for the genus Stenophora. I am able to offer nine stages, somewhat arbitrarily chosen, in the development of a single species, the species chosen being *Stenophora lactaria* from the milliped *Callipus lactarius* (Say), described by the writer (1915: 29-30; 1916; 72-4). The intestines of several hosts were removed intact, fixed with corrosive-acetic, and sectioned. Sections were cut 4μ thick and stained with Ehrlich's hematoxylin. All the intestines proved heavily infected. The lumen reveals parasites in the proventriculus and in the large intestine, but intracellular stages are generally lacking except in the first-named portion.[1] In several instances parasites were found boring through the walls into the coelom. Successive movements have been traced from the penetration of the muscular layer of the digestive tract

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[1] The digestive tract of the milliped (Fig. 12) is, according to Leidy (1853), divided into (*a*) and (*b*) six salivary glands, (*c*) esophagus, (*d*) proventriculus, (*e*) two bile ducts, (*f*) broad opaque cuticular curtain, (*g*) ventriculus, (*h*) large intestine, (*i*) rectum.

through the coelomic epithelial layer until the parasites are free in the coelom. This phenomenon was reported by the writer in a former paper (1916: 29). The gregarine possesses no boring apparatus; it has no chitinous style and it even lacks an epimerite; thus, mechanical apparatus being absent, the hypothesis is made that the means used is chemical and that the body of the parasite secretes a fluid destructive to the cell epithelium. The cells in the immediate vicinity of the tubular opening are crowded back and disorganized and their nuclear material scattered. Both the nuclear and cytoplasmic protoplasm stain less deeply than the normal. This fluid present is either a secretion of the parasite available for purposes of penetration or the normal excretion of the parasite which is toxic to the adjacent tissues.

After the cyst has been discarded from a host along with its feces and has dehisced, the spores are liberated and accidental parasitism occurs, the released spores being eaten by a host of the same or a nearly related species,[2] even by the original host. The spore, upon reaching the alimentary canal, loses its sporocyst by the action of the digestive juices, releasing, in all the Eugregarinae, eight falciform sporozoites.

The stages in development from the incipient to the mature parasite which have been studied are as follows:

The liberated sporozoite, which is slightly motile, although it possesses neither cilia nor flagella, reaches the epithelium of the proventriculus and penetrates between the terminal cilia into the free end of one of the absorptive cells (Fig. 1). The sporozoite can be readily detected, although less than 5μ in length, because of its intense coloration, staining darker than the cytoplasm of the cells. A nucleus can be discerned as it is still darker. The sporozoite loses its falciform shape and rounds off at one end, penetrating the cell by the remaining pointed extremity.

It journeys down the cell past the nucleus, the pointed end preceding (Fig. 2).[3] The parasite is small enough to make the passage without injuring the cell other than by the temporary crowding of the nucleus.

It comes to rest at the end of the cell, next the muscular layer (Figs. 3 and 4, a). The sporozoite now becomes trophic, viz., a *trophozoite*. With the beginning of growth and consequent excretion, the parasite effects a chemical change upon the parasitized cell. The cytoplasm becomes slightly vacuolated and stains a little less deeply than normal, but its nucleus is not yet visibly affected.

In the next stage, the parasite has lost its characteristic sporozoite shape and has become a subspherical body of larger dimensions and

[2] Léger and Duboscq (1902) have shown that if the spore is eaten by any other animal, it will not dehisce but passes intact through the digestive tract.

[3] Léger and Duboscq record that in *Stenophora aculeata* the sporozoite pushes the cell nucleus ahead of it toward the muscular layer gradually absorbing it.

with a conspicuous nucleus containing one karyosome, which is characteristic of the adult nucleus (Fig. 4, b). The host cell has become still further vacuolated and stains still less deeply. The nucleus is now affected, for it, too, stains more faintly.

The trophozoite now becomes differentiated into protomerite and deutomerite separated by a septum (Fig. 4, c). The nucleus and karyosome have grown more rapidly than the parasite and are proportionally much larger than before. It is already apparent that the protomerite stains more intensely than does the deutomerite. This continues into the mature sporont stage. The young trophozoite generally orients itself so as to lie "head" downward, i. e., with the protomerite next the muscular layer, but exceptions occur. In some instances also the parasite lies at right angles to its usual position or in the antero-posterior plane of the host. In a few instances out of hundreds, the protomerite was seen to be directed toward the lumen. Léger and Duboscq record this in *Stenophora aculeata* (1904), and Mercier in *Cephaloidophora talitri* (1912). The former authors name the two possibilities, viz., that the sporozoite penetrated the cell posterior end first or else turned after entrance. The cytoplasm of the cell is still further destroyed. It may be vacuolate with the nucleus intact and seemingly but little changed (Fig. 5) or vacuolate with the nucleus already destroyed, its remaining chromatin massed at the base (Fig. 4, c).

The protoplasm of the two divisions of the trophozoite is becoming differentiated (Fig. 6). In the protomerite it stains deeply and consists of small homogeneous granules, while in the deutomerite it is more coarsely granular and less closely packed together. The nucleus of the parasite has now begun to assume the shape of that in the adult sporont; it has become ellipsoidal and is now smaller in proportion to the size of the gregarine than it has been before. The protomerite has acquired a papillate apex which is retained in the adult. This is the only structure in this species which may be compared to an epimerite. It is not a true epimerite, for it performs no function. It may possibly be a vestigeal remnant from a lower group of gregarines which possesses and uses a true epimerite, but the relationship of the families of gregarines has been little discussed and the higher or lower position of the Stenophoridae is not determined. Some members of the genus appear to possess epimerites, as *S. nematoides* Léger and Duboscq (1904) and *S. dipolcorpa* Watson (1916); one species retains a minute apical style (*S. aculeata* Léger and Duboscq (1904)); but most species have no trace of an epimerite at any stage of development.

The parasite has by this time acquired something of the normal sporont shape (Fig. 7). It has grown so as to absorb several adjoining cells besides that first parasitized, nuclear and cytoplasmic vestiges

remaining at the base at one side of the apex of the protomerite. By growth and expansion the animal has laterally compressed the cells which border it, leaving a small opening into the lumen of the alimentary tract. It is my opinion that a part of the parasite's nourishment is acquired by absorption direct from the intestine through this opening.

The stage shown in Figure 8 is very similar to the last. The space leading to the lumen is larger and the contiguous cells have been forced farther apart with a consequent compression of many cells, their subspherical nuclei having become very long and slender. It is to be noted that the deutomerite is growing faster than the protomerite and is forcing itself down over the former at the septum, while the protomerite is flattened against the muscular layer.

The trophozoite has become capable of living free in the intestinal lumen (Fig. 9). It no longer receives sufficient nourishment from the epithelium and through the small opening into the lumen, and is forced out henceforth to lead a free existence in the lumen. Just what forces it out of the epithelium, I am unable to say. One would not be inclined to assume in it the power of volition with the ability to leave the cell at the critical moment; but, on the other hand, one cannot assume that the cells force it out by swelling and expressing it, for up to now they have been passively forced apart by the growing parasite.

By whatever means, the animal has left, after absorbing nourishment from many cells which are not entirely destroyed but only distorted with their nuclei shrivelled. These cells are probably able to revive themselves and acquire new vigor, unlike the first few cells, which were totally destroyed. The animal has not straightened itself out yet from the cramped position when embedded, and the deutomerite still overlaps the protomerite. The liberated trophozoite has become a free living *sporont.*

The young sporont (Fig. 10) must now receive all its nourishment from liquids in the alimentary tract and not indirectly through the media of cells. The animal is rotund in appearance and sluggish; the epicyte is seen to be thicker at the septum than elsewhere; the papillated apex of the protomerite is apparent, and there is visible for the first time a minute indentation in this apex which is frequently reported for this genus.

The fully developed sporont is much more graceful (Fig. 11). The deutomerite has grown more rapidly than the protomerite, leaving the latter a small conoidal segment while the latter has elongated and become slender and tapering. The nucleus acquired its permanent shape in an early stage, and now remains elongate-ellipsoidal with one large karyosome.

It is seen that in the species considered there is no epimerite. Nourishment, then, takes place by absorption from surrounding cells directly through the epicyte of the parasite. An epimerite would, moreover, be superfluous to an animal which is intracellular in development, useful only in a species in which one end only of the parasite is embedded in an epithelial cell.

The parasitized cell is apparently unaffected until the sporozoite has begun to grow. Then the cytoplasm becomes reticulate, vacuolate, and resistant to the staining fluid. It commences to shrivel in length and in width; atrophy has set in. This may be due to the toxic influence of the parasite, but it is undeniably due in part at least to the absorption of its vital fluids by the rapidly growing parasite. No hypertrophy is noted from the first. The nucleus is affected less readily than the cytoplasm, but soon shows a resistance to the stain. For a time the chromosome count is unaltered, although the size of the individual chromosomes is reduced. The growing animal utilizes the space left by the contracting cell and compresses adjoining cells in its proximity, while the cells become elongate and wider at the uncompressed ends. The posterior end of the gregarine soon projects into the lumen, for it forces the cells so far apart that they are no longer able to overlap the end of the parasite. The latter now undoubtedly receives some of its nourishment from the lumen and the drain on the cells themselves is decreased. This probably accounts for the fact that frequently not more than two or three cells are actually destroyed, the others being compressed only during the occupancy of the parasite. When the latter departs, the cells return to something like their former shape, and the space through which the parasite left almost immediately closes over.

The cells surrounding the parasite always are separated a trifle from it, leaving in many instances a thin clear area around it. I think this is due to the fact that in fixing the parasite shrinks slightly more than does the host tissue, rather than to the fact that the parasite affects the cells toxically and causes them to draw back.

The cells of the proventriculus lie in lobes (Figs. 1, 4, 7, and 9), the deep-seated lobes being the ones generally parasitized. Only in unusual cases do the outlying ones harbor gregarines. The deeper seated cell forms a safe harbor for the young sporozoite where it is not in danger of being swept along in the lumen by the undigested food masses and by the animal's movements. These shorter cells also afford easy exit when the parasite is ready to leave the epithelium, being about as long as the mature trophozoite when it leaves.

The trophozoite always lies next the basement muscular layer, but not actually in contact with it; it never remains half way up the cell. As aforesaid, it is usually placed "head downward."

That the gregarine is intra-cellular rather than inter-cellular is seen from the early stages when the cell itself contains the parasite; with later stages alone this could not be determined.

Several writers have noted that in the species which they studied distinct hypertrophy of the parasitized cell occurred. Laveran and Mesnil (1900) and Léger and Duboscq (1909a) have shown that the parasitized cell decreased in length, but at the same time increased abnormally in width due to the influence of toxic excretions of the parasite. The latter authors think the cell is not killed, but assumes its normal shape and function after being relieved of its burden. They state, however, that the influence of the parasite upon the host cells is very different in different hosts, with different parasites, and even in different parts of the same host.

The present research has shown that there is no hypertrophy of the cell, but that the originally parasitized cell shrinks from the start without widening and is destroyed, and that the two adjoining cells are in many instances also destroyed. Other nearby cells are temporarily compressed and elongated, later to return to their normal functions; their staining reaction is unaffected.

SUMMARY

Consecutive stages in the growth of *Stenophora lactaria* Watson are depicted.

This species does not possess an epimerite.

Development is intracellular and the parasitized cell is destroyed.

No hypertrophy is indicated at any stage of development.

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Explanation of Plates 1 and 2

Fig. 1.—Sporozoite (*s*) beginning to penetrate an epithelial cell of the intestine.

Fig. 2.—Sporozoite (*s*) ascending the cell.

Fig. 3.—Sporozoite (*s*) at rest at the base of the epithelial cell.

The line at the right represents 50 μ.

Fig. 4.—Three stages in the growth of the parasite: (*a*) same as shown in Fig. 3; (*b*) trophozoite with enlarged nucleus and one karyosome; (*c*) larger trophozoite with formed septum, the cell nucleus destroyed and cytoplasm somewhat vacuolated. The line at the right represents 50 μ.

Fig. 5.—Still larger trophozoite with cell nucleus yet intact altho the cytoplasm is affected. The line represents 25 μ.

Figs. 6, 7, and 8.—Stages in the growth of the trophozoite, cell nuclei in each instance destroyed, vestiges remaining at the "head" of the parasite. The line represents 50 μ.

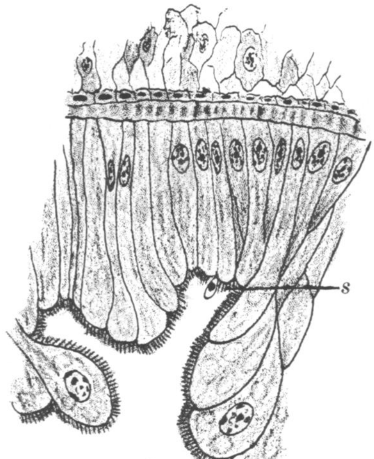
Fig. 9.—Trophozoite leaving the epithelium and migrating into the intestinal lumen. The line represents 50 μ.

Fig. 10.—Sporont soon after emerging and still rotund in appearance.

Fig. 11.—Mature sporont from lumen showing proportional growth of protomerite and deutomerite.

Fig. 12.—A copy of Leidy's figure (1853, Plate VII, Fig. 21) of the digestive tract of *Julus marginatus*.

PLATE 1



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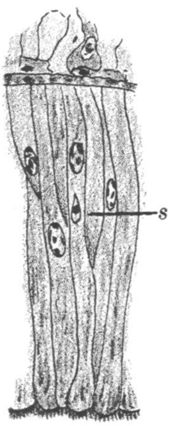

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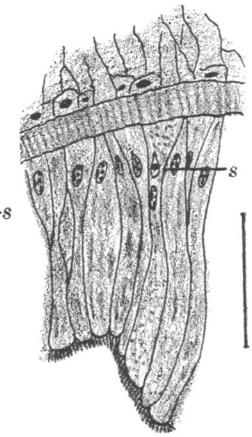

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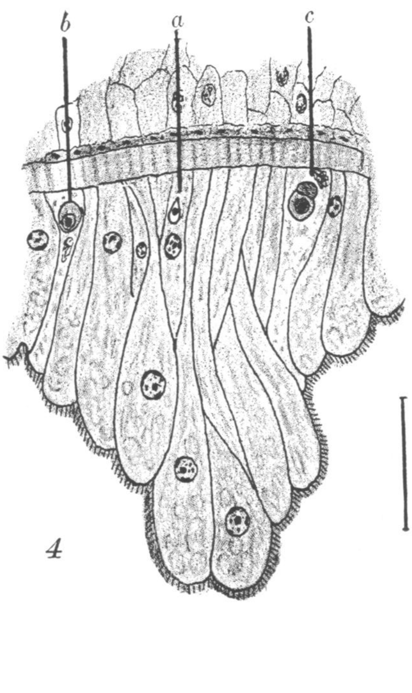

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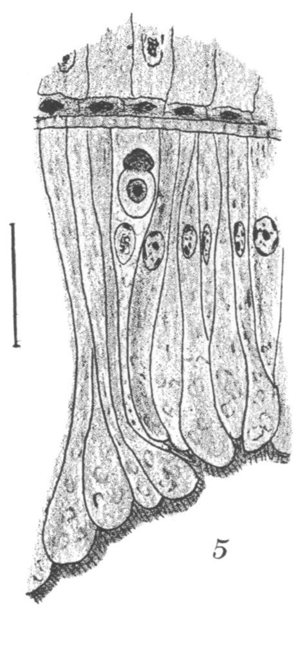

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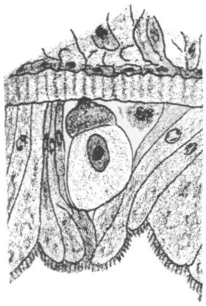

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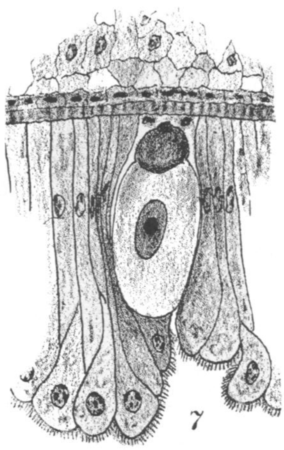

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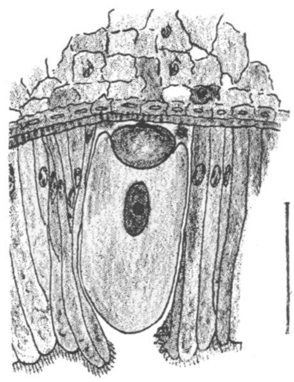

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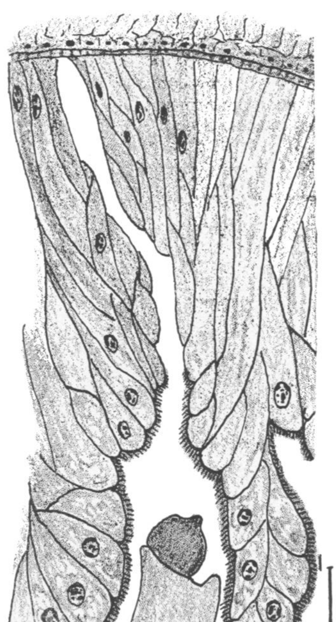

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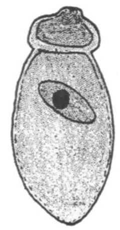

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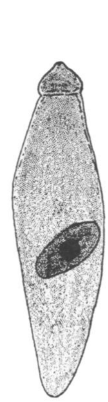

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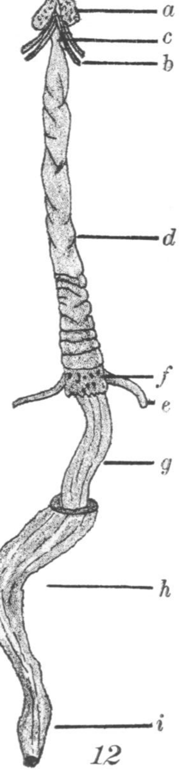

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